International Journal of
Cosmetic Science

International Journal of Cosmetic Science, 2019, 1–7 doi: 10.1111/ics.12534

Attenuation of negative effects of senescence in human skin using an extract from *Sphingomonas hydrophobicum*: development of new skin care solution

S. Gervason*, M. Napoli*, A. Dreux-Zhiga*, C. Lazzarelli†, S. Garcier†, A. Briand†, M. Albouy‡, A. Thepot‡, J. Y. Berthon* and E. Filaire*§

*GREENTECH SA, Biopôle Clermont-Limagne, 63360, Saint-Beauzire, France, †Deinove, 1682 rue de la Valsiere, 34790, Grabels, France, ‡Labskin Creations, Hôpital Edouard Herriot Bâtiment, 5 Place d'Arsonval, 69003, Lyon, France and §INRA, UNH, Unité de Nutrition Humaine, CRNH Auvergne, Université Clermont Auvergne, F-63000, Clermont-Ferrand, France

Received 6 March 2019, Accepted 23 April 2019

Keywords: ageing, psychobiology, self-esteem, senescence, skin, *Sphingomonas*

Abstract

OBJECTIVE: Intrinsic skin ageing is mainly caused by cellular senescence. p16 and p21 are two important tumour suppressor proteins that play essential roles during cell proliferation and ageing through regulating the expression of several genes. Moreover, physical changes between the ages of 55 and 60 years affect one's physical and disrupt self-esteem. The cosmetics industry has focused on bioactive substances derived from natural products such as plants, mushrooms and marine algae to counteract the deleterious effect on skin senescence. Besides these products, compounds produced by bacteria may decelerate individual senescence.

METHODS: In order to evaluate the potential benefits of bacteria extract over skin ageing, we investigated whether a *Sphingomonas hydrophobicum* (SH) extract is able to protect our skin against senescence mechanisms. We used an ageing full-thickness skin equivalent model, which was treated or not with the bacteria extract in a systemic way for 42 days. p21 and p16 and senescence-associated galactosidase activity were used to detect cellular senescence with immunohistology. Using a psychobiological approach, we evaluated *in vivo* the effect of SH extract on self-esteem, isotropy and suppleness.

RESULTS: *Sphingomonas* extract significantly suppressed senescence associated with β-galactosidase activation. It also significantly inhibited the expression of cell cycle inhibitors (p21 and p16). At the same time, the bacteria extract has a significant positive impact on the issue by increasing the expression of versican and fibrillin-1. Significant improvements of self-esteem were reported after 56 days of SH extract application. These psychological benefits were accompanied by a significant improvement in skin suppleness and isotropy.

CONCLUSION: *Sphingomonas* extract delays intrinsic skin ageing process by inhibiting cellular senescence, and has also the capability to restructure the skin. These beneficial physiological effects induced by SH extract have a positive influence on self-esteem. Because skin ageing causes emotional distress, SH extract can serve as an anti-ageing cosmeceutical agent and help to build a better psychological health, and help individuals to assume ageing.

Correspondence: Edith Filaire, GREENTECH SA, Biopôle Clermont-Limagne, 63360 Saint-Beauzire, France. Tel.: 0473939900; fax: 0473939932; e-mail: edithfilaire@greentech.fr

Résumé

OBJECTIF: Le vieillissement intrinsèque de la peau est principalement causé par la sénescence cellulaire. p16 et p21 sont deux importantes protéines suppressives de tumeurs qui jouent un rôle essentiel dans la prolifération et le vieillissement cellulaire en régulant l'expression de plusieurs gènes. De plus, les changements physiques survenant entre 55 et 60 ans affectent le physique et perturbent l'estime de soi. L'industrie cosmétique s'est concentrée sur les substances bioactives dérivées de produits naturels tels que les plantes, les champignons et les algues marines pour contrer les effets délétères sur la sénescence de la peau. En plus de ces produits, les composés produits par les bactéries peuvent ralentir la sénescence individuelle.

MÉTHODES: Afin d'évaluer les bénéfices potentiels de l'extrait de bactérie sur le vieillissement cutané, nous avons étudié si un extrait de *Sphingomonas hydrophobicum* (SH) est capable de protéger notre peau des mécanismes de sénescence. Nous avons utilisé un modèle équivalent de peau vieillissante de pleine épaisseur, qui a été traitée ou non avec l'extrait de bactérie de façon systémique pendant 42 jours. p21 et p16, et l'activité galactosidase associée à la sénescence ont été utilisés pour détecter la sénescence cellulaire par immunohistologie. En utilisant une approche psychobiologique, nous avons évalué in vivo l'effet de l'extrait de SH sur l'estime de soi, l'isotropie et la souplesse.

RÉSULTATS: *L'extrait de* Sphingomonas a considérablement supprimé la sénescence associée à l'activation de β-galactosidase. Il a également inhibé de manière significative l'expression des inhibiteurs du cycle cellulaire (p21 et p16). En même temps, l'extrait de bactérie a un impact positif significatif sur le problème en augmentant l'expression du versican et de la fibrilline-1. Des améliorations significatives de l'estime de soi ont été rapportées après 56 jours d'application de l'extrait de SH. Ces bienfaits psychologiques s'accompagnaient d'une amélioration significative de la souplesse et de l'isotropie de la peau.

CONCLUSION: *L'extrait de Sphingomonas* retarde le processus de vieillissement intrinsèque de la peau en inhibant la sénescence cellulaire et a également la capacité de restructurer la peau. Ces effets

physiologiques bénéfiques induits par l'extrait de SH ont une influence positive sur l'estime de soi. Parce que le vieillissement de la peau provoque une détresse émotionnelle, l'extrait de SH peut servir d'agent cosméceutique anti-âge et aider à construire une meilleure santé psychologique, ainsi qu'aider les individus à assumer le vieillissement.

Introduction

Cellular senescence is an irreversible growth arrest that occurs further to different stimuli, including oxidative, epigenetic and genotoxic stresses, telomere shortening and mitochondrial dysfunction. They are characterized by the inability to proliferate, resistance to apoptosis, activation of oncogenesis and secretion of factors involved in inflammation and tissues deterioration [1]. Even though senescent cells had beneficial functions in wound healing, their accumulation with age negatively affects the surrounding tissue [2].

The initial step of senescence represents the progression from a transient to a stable cell cycle arrest through sustained activation of the p53-p21 or/and p16-Rb pathways. The resulting early senescent cells progress to full senescence by downregulating lamin B1, thereby triggering extensive chromatin remodelling underlying the production of a senescence-associated secretory phenotype (SASP). Some components of the SASP are highly conserved, whereas others may vary depending on cell type, nature of the senescence-inducing stressor or cell to cell variability in chromatin remodelling. SASP comprises a complex mix of factors including cytokines, growth factors, chemokines and matrix metalloproteinases. Generally speaking, the SASP is responsible for many of the positive and negative functions attributed to senescent cells [3]. On one hand, the major function of the SASP is to recruit the immune system to eliminate senescent cells. Secretion of MMPs and factors such as VEGF can remodel the surrounding tissue, inducing angiogenesis and reducing fibrosis. On the other hand, secretion of molecules such as TGF-β can spread the senescence phenotype in a paracrine manner to surrounding cells [4]. Factors secreted by senescent cells can reinforce the senescent phenotype, potentially exacerbating senescence during ageing. IL-8, IL-6 and IGBP-7 are among the specific SASP components reinforcing senescence. Recently, it has been noted that senescent cells accumulating in response to tissue damage can also promote reprogramming [5].

The identification of markers that unequivocally detect and quantify senescent cell remains in debate. Nevertheless, elevated β-galactosidase activity is related to the increase of lysosomal activity of SA-β-gal. Other molecular markers such as p16, p21, expression of secretion factors (IL-6, IL-8) and changes in chromatin (HP1, Hira) have been identified in the characterization of the senescent state [6]. The use of such markers has provided convincing evidence that senescent cells indeed accumulate in tissues of humans and rodents with age, skin ageing inducing visible signs such as thin and dry skin, wrinkles, degradation and structural alterations of extracellular matrix. Generally speaking, aged skin becomes atrophic due to reduced proliferation, increased inflammation and breakdown of collagen fibres.

Besides deleterious effects on physiological parameters, ageing affects psychological state. In fact, it has been shown that ageing has negative effect on mood and self-esteem. It is also seen that many woman attaches self-esteem to their body image which is associated with beauty, femininity and youth so with growing age as beauty diminishes many a woman find their self worth low and thus giving rise to a low self-esteem [7]. Today, it is no longer a rarity to live more than 90 years, individual expecting to live longer and in good health, without deleterious effects associated with ageing. This status is referred to as ageing well. From this trend, a new dermo-cosmetic concept emerged, well ageing, which focuses on wellness in order to maintain health capital. This concept is linked to the psychophysiological approach.

Since a long time, identified senescent cells have been performed on *in vivo* skin analysis, which contains between 20% and 60% of senescent fibroblasts. Interfering with senescent cells seems to be beneficial for the health and the development of specific interventions that target senescent cells serving as a therapeutic approach to delay ageing and skin pathologies [6]. The cosmetics industry has focused on bioactive substances derived from natural products such as plants, mushrooms and marine algae. Besides these products, bacteria such as *Lactococcus lactis* strain may prevent immune senescence [8]. The genus *Sphingomonas,* hallmarked by their oligotrophic nature and plasticity in man-made environments, has been intensively exploited for their metabolic properties relevant to biotechnological importance [9]. *Sphingomonas strains* belong to the proteobacteria phylum and are a Gram-negative, chemoheterotrophic and strictly aerobic bacteria [10]. They frequently exhibit a yellow pigment coloration due to the presence of two enzymes, a catalase and an oxidase, which allow it to produce a carotenoid pigment named nostoxanthin. The precise function of this unique carotenoid in this type of microorganisms is likely associated with tolerance to environmental stress due to the antioxidant activity of carotenoids. The second specificity of these bacteria is to contain glycosphingolipids (GSLs) instead of lipopolysaccharide (LPS) in their cell envelopes as observed in other Gram-negative bacteria. The GSLs appear also to act as a barrier to bactericidal substances [11]. During the past 10 years, *Sphingomonas* strains have been isolated from a variety of environments including both aqueous (both fresh and seawater) and terrestrial habitats and plant root systems [12]. The widespread distribution in the environment is due to its ability to utilize a wide range of organic compounds and to grow and survive under low-nutrient conditions. Due to their biodegradative and biosynthetic capabilities, these bacteria have been used for a wide range of biotechnological applications, from bioremediation of environmental contaminants to production of extracellular polymers such as sphingans (e.g. gellan, welan and rhamsan) used extensively in the food and other industries [10]. These bacterial species described in the environment may play a role in skin homoeostasis. This is the One-Health concept, which recognizes that the health of humans is connected to the environment [13].

Based on its rich and unique composition, *Sphingomonas strains* represent an innovative source for the development of new skin care solutions. In this study, we firstly aimed to investigate the effect of *Sphingomonas hydrophobicum* (SH) extract on the expression of p16, p21 and SA-β-gal activity using an aged 3D human skin model, a full-thickness skin model engineered with aged fibroblast treated during the tissue reconstruction. Based on the beneficial properties of microorganisms and the plasticity of bacterial genomes allowing bacteria to adapt quickly to environmental conditions, we put forward the hypothesis that *Sphingomonas hydrophobicum* extract could slow down the cell senescence mechanism. Secondly, we evaluated the effect of SH extract on the elasticity of the dermal compartment by the analysis of the expression of the fibrillin and versican proteins. Finally, using a psychobiological approach, we evaluated *in vivo* the effect of SH extract on self-esteem, and biomechanical

properties of the skin. *Sphingomonas hydrophobicum* contains glycosphingolipids acting as a barrier to bactericidal substances. We put forward the hypothesis that SH not only imparted anti-ageing effects but also has a synergistic sense of emotion.

Materials and methods

Bacterial strains and culture conditions

Sphingomonas hydrophobicum was isolated from water samples of a site located in Tartras (Aquitaine, France) and has been kept in the Deinove's collection (Montpellier, France). PGY agar plate was used to isolate the strain (/L): 5 g of Tryptone, 1 g of D-Glucose, 3 g of yeast extract, 15 g of agar.

Sphingomonas hydrophobicum was cultivated on Complex Medium Glucose (CMG):10 g L^{-1} of glucose, 5 g L^{-1} of yeast extract (YE), 2 g L^{-1} of bacto-peptone, 5.74 mM K_2HPO_4, 10% v/v 3-(N-morpholino) propanesulphonic acid (MOPS) buffer mixture (400 mM MOPS, 200 mM NH_4Cl, 100 mM NaOH, 100 mM KOH, 2.76 mM Na_2SO_4, 5.28 mM $MgCl_2$ and 5 μM $CaCl_2$), The MOPS buffer mixture added was sterilized by filtration. The initial pH was adjusted to 7 with NH_4OH. Culture conditions were defined as follows: temperature 30°C, pH 7, in rotary shaker at 250 rpm during 21 h.

Starting culture of *Sphingomonas hydrophobicum* was used to inoculate a bioreactor containing 20 L of Complex Medium Glucose (CMG). Culture conditions were defined as follows: temperature 30°C, pH 7, impellor speed from 300 to 900 rpm and the aeration rate between 0.25 to 1 vvm. A batch strategy of 48 h of fermentation was performed to achieve the highest yield of biomass using a 5% v/v inoculum.

After 48 h of production, the culture was centrifuged to recover the bacterial cells with a dry matter of approximately 15% (m/m). The recovered biomass was mixed with ethanol 96% at ratio of 10 : 1 (ethanol : biomass (m/m)), heated at 60°C during 30 min and then incubated at room temperature for 30 min. The extract obtained was filtered on filter paper, mixed in propanediol and placed in a rotary evaporator until ethanol was evaporated. Finally, the extract is filtered at 0.2 μm and dry matter is adjusted to obtain 2% (m/v).

MTT cell viability assay

To evaluate the effect of our bacterial extract on senescence parameters, we used an ageing full-thickness skin equivalent model with 57-year-old Normal Human Dermal Fibroblasts (NHDF) and treated with the bacterial extract at different concentrations, in a systemic way for 42 days. As a preliminary evaluation, cytotoxic analysis on cell culture monolayers was conducted to select the highest non-cytotoxic concentrations and to avoid any cumulative deleterious effect on 3D reconstructed skin model.

The cytotoxicity using a 5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assays on primary keratinocytes and fibroblasts cultured in monolayer was performed for 7 days to evaluate the direct or cumulative cytotoxic effect of the *Sphingomonas* strain. The cells used for the cytotoxicity tests were the same as those used for the reconstructed skin, namely NHDF and Normal human epidermal keratinocytes (NHEK). NHEK and NHDF were trypsinized and seeded into 48- or 96-well plates, respectively, at a density of 10 000 cells/well and cultured to confluency. *Sphingomonas extract* was then put in contact with the cells for 7 days before performing the MTT assay (incubation with MTT at 0.5 mg mL^{-1} for 3 h and dissolution of the formazan crystals with 100 μL of DMSO under stirring for 30 min). For each condition, sixplicate was performed for each cell type. After reading the absorbance of the plates at 550 nm, the percentage of viability of each condition was calculated and normalized to the untreated control, representing 100% viability.

The cytotoxicity tests were carried out on five different concentrations of *Sphingomon*as extracts and its corresponding solvent as a control. The assay was validated since SDS 0.5% induced a viability lower than 20%. We observed that the first non-cytotoxic concentration was 0.1%. We noted that the ingredient cytotoxicity is very close to its solvent cytotoxicity. For keratinocytes, the first non-cytotoxic concentration was 0.05%. Higher concentrations caused significant cell mortality.

Reconstructed skin model

We used a full-thickness skin based on a scaffold made of collagen–glycosaminoglycan–chitosan. Primary NHDF were seeded at D0 in the dermal substrate. The dermal equivalent was then grown in immersion for 21 days. On day 21, keratinocytes from young donor (3y.o) were seeded of the top of the dermal equivalent. This skin equivalent was grown on immersion 1 week before being raised at the air–liquid interface, enabling a complete differentiation and maturation of the epidermis. The reconstructed skin samples were treated with *Sphingomonas extract* dissolved in the cell culture medium from day 3 until day 42. The study was conducted in comparison with the reconstructed skin sample grown in normal medium without active ingredient (untreated control).

Immunohistological analysis

Several immunohistological analyses were realized to study the elasticity of the dermal compartment by the analysis of the expression of the fibrillin and versican proteins. Finally, the fibroblast senescence was investigated by the analysis of the p16, p21 expression and *β*-galactosidase.

Immunofluorescence on OCT-embedded frozen samples**:** labelling was performed on air-dried 5 μm cryosections. After 10 min fixation with glacial acetone, sections were incubated in PBS containing 4% of Bovine serum albumin for blocking non-specific binding. Sections were then incubated with the following primary antibodies: Fibrillin (Thermofisher, Bourgain-Jallieu, France) and Versican (Novus Biological, Centennial, CO, USA).

Immunohistochemistry on paraffin-embedded formalin-fixed samples: paraffin-embedded formalin-fixed samples were cut into 5 μm sections. After heat-mediated antigen retrieval treatment, tissue sections were incubated in 5% H_2O_2/3% normal goat serum (NGS) (Jackson Immunoresearch, Suffolk, U.K.) to inactivate endogenous peroxidases. Non-specific binding was blocked in PBS containing 5% of NGS. Sections were then incubated with the following primary antibodies diluted in PBS/NGS 5% overnight at room temperature (Ventana medical System for p16, Abcam for p21). For immunohistochemical detection of p16, signal was revealed following the supplier's instruction (CINtec® Histology kit, ref 06594441001, Roche, CA, USA). Tissue sections were subsequently counterstained using Harris' haematoxylin (25%, Sigma Aldrich, Saint-Quentin Fallavier, France). As a negative control, the corresponding IgG class replaced primary antibody. For immunohistochemical detection of *β*-Galactosidase, 5 μm cryosections were fixed for 1 min with paraformaldehyde 1%. They were

then incubated during 12–16 h at +37°C in a staining solution included 1 mg mL^{-1} of β-Galactosidase distilled in 10 mL of water, 40 mL of citric acid/sodium phosphate solution, 10 mL of potassium hexacyanoferrate, 10 mL of potassium hexacyanoferrate trihydrate, 6 mL of sodium chloride, 400 μL of magnesium chloride and 123.6 mL of distilled water.

After these steps, sections were rinsed twice with PBS and counterstained with Eosin 0.5% for 2 min.

Image acquisition: Immunostainings and histological stainings were observed using an Axio observer D1 optical microscope (Zeiss). Images were captured using an HRc AxioCam camera and ZEN 2 blue software (Zeiss, Louvain La Neuve, Belgium) and saved in an uncompressed tagged image file format (tiff). Image processing and analysis were performed using the software ImageJ (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, MA, U.S.A., http://imagej.nih.gov/ij/, 1997–2017). Between 6 and 10 images were analysed for each condition. The parameters in which we were interested were the neo-synthetized extracellular matrix for Masson's Trichrome-stained sections, p16, p21, and the surface area of versican and fibrillin-1.

Pixels corresponding to the markers of interest were segmented from other pixels. p16 and p21 nucleus-positive epidermal cells (brown staining) were automatically detected and separated from the background and negatively stained cells by watershed segmentation. Data are expressed in number of positive cells per epidermal field. For the surface measurement, the area of interest was measured automatically and segmented from the other pixels. Data were normalized by the area of the dermis. Results were expressed in area fraction (arbitrary unit).

In vivo study

The study was performed in open, intra-individual study by comparison before and after the application of SH extract formulated at 1% vs. placebo, twice a day. Twenty-four subjects were involved from 60 to 70 years old. The study was performed during 56 days.

The inclusion criteria were: person with loose skin on the face, person with wrinkles and fine lines on the face, subject with dry skin on the face (cutaneous hydration rate < 70 A.U.) on jaws, checked using Corneometer®.

The exclusion criteria were: cutaneous pathology on the face, subject having used on the studied zone, an anti-wrinkle product (or product in action on the skin's surface) or having stopped it for less than 1 week, subject having done facial injections and/or a palpebral lifting, use of topical or systemic treatment during the previous weeks liable to interfere with the assessment of the cutaneous acceptability of the studied product.

Isotropy

Orientation of the lines in the cutaneous relief, allowing the evaluation of the restructuring effect on cheek using 3D PRIMOS® Lite, was measured at the beginning and after 56 days of SH extract treatment. This technique consists in calculating a phase image from images with interference fringe projection. The acquisition software allows to obtain 2D and 3D measurements. The parameter measured is the isotropy, which defines the orientation of the lines in the cutaneous relief. In fact, the ageing mechanism results in a change in the organization of skin lines passing of an homogeneous isotropic state (oriented lines in all directions) for a young person to a state where small furrows gradually disappear to give rise to a state where only persist deep furrows leading to the formation of wrinkles (privileged directions).

Biomechanical properties of the skin

They were evaluated using a Cutometer®, which is an *in vivo* non-invasive method to evaluate skin rheological properties: measures of biological extensibility and elasticity variations. The technique consists on the suction of the skin in the orifice of a probe by a constant vacuum pressure and for a constant duration. The depth of penetration of the skin into the probe is measured, without friction and mechanical effects, by using two optical prisms located at the opening of this probe. Cutaneous skin elasticity is performed with a 2 mm or a 6 mm probe, depending of the measured zone, with one cycle of measurement and a 450 mbar constant pressure (for 2 mm probe) or 350 mbar (for 6 mm probe). Suction and relaxation times are of 3 s. Measurements were done at the beginning and at after 56 days of SH extract application.

Psychological parameter

Self-esteem was investigated using the Rosenberg test, which is a widely used self-report instrument [14]. It is a 10-item scale that measures global self-worth by measuring both positive and negative feelings about the self. All items are answered using a 4-point Likert scale format ranging from strongly agree to strongly disagree. It appears that self-esteem has a strong relation to happiness. The measure demonstrated good internal consistency (Cronbach's alpha reliability coefficients was 0.85), consistent with published estimates [15]. This evaluation was carried out at Day 0 and Day 56.

Statistics

All statistical analyses were performed using the SPSS program. All values are expressed as mean ± SD. Mann–Whitney *U* test was performed because of non-Gaussian distributions. P value < 0.05 was considered statistically significant.

Results

Sphingomonas decreases the expression of senescence-related proteins

In Fig. 1, we noted a significant decrease (−22%; $P < 0.001$) in the expression of p16 when compared to the untreated condition. *Sphingomonas* extract treatment also allowed to decrease of p21 expression (69%: $P < 0.001$) when compared to control condition.

Sphingomonas decreases the expression of β-galactosidase in the dermis

Sphingomonas extract treatment at 0.1% concentrations leads to a decrease of β-galactosidase expression as compared to the untreated control. This decrease was about 69.4% ($P < 0.01$) (Fig. 2).

Expression of fibrillin-1 and versican

Figure 3 shows the expression of fibrillin-1, the main component of microfibrills in the dermal extracellular matrix. The fibrillin-1

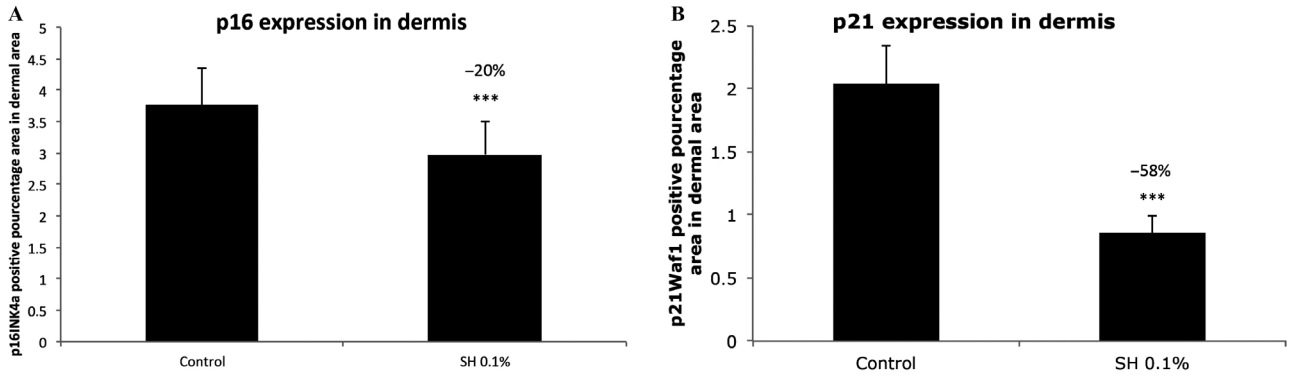


Figure 1 (A) p16 expression quantification normalized by the total dermal area. Automatic analysis of staining with "ImageJ" software. ***$P < 0.001$ vs. control. (B) p21 expression quantification normalized by the total dermal area. Automatic analysis of staining with "ImageJ" software. ***$P < 0.001$ vs. control.

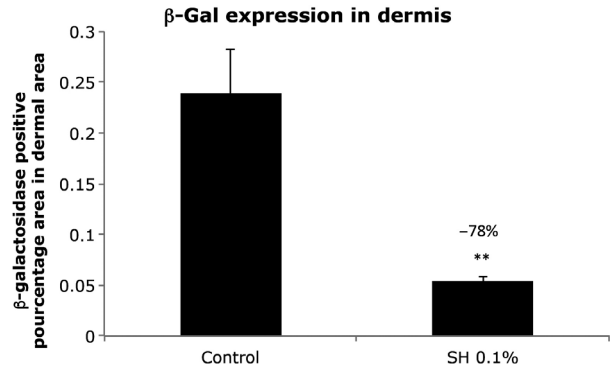


Figure 2 Comparison between the expression of β-Galactosidase in dermis after SE treatment as compared to control. Mean ± SD. **$P < 0.01$.

participates in the formation of elastic fibres and also plays a role in the regulation of different cytokines and growth factors. Our study showed that treatment with *Sphingomonas* extract increased significantly the fibrillin-1 expression in the dermis of reconstructed skin (22%: $P < 0.001$). This treatment also induced a significant increase in versican expression (7%; $P < 0.05$).

In vivo results

After 56 days of application, SH extract induced a significant improvement in isotropy (+14%: $P < 0.02$). At the same time, the application of placebo induces an isotropy decrease.

We also noted a significant increase in Ue parameter (reflecting the suppleness evaluated using the deformation and immediate extensibility of the skin) of +12% ($P < 0.001$). At the same time, the placebo induces a non-significant decrease of 4%.

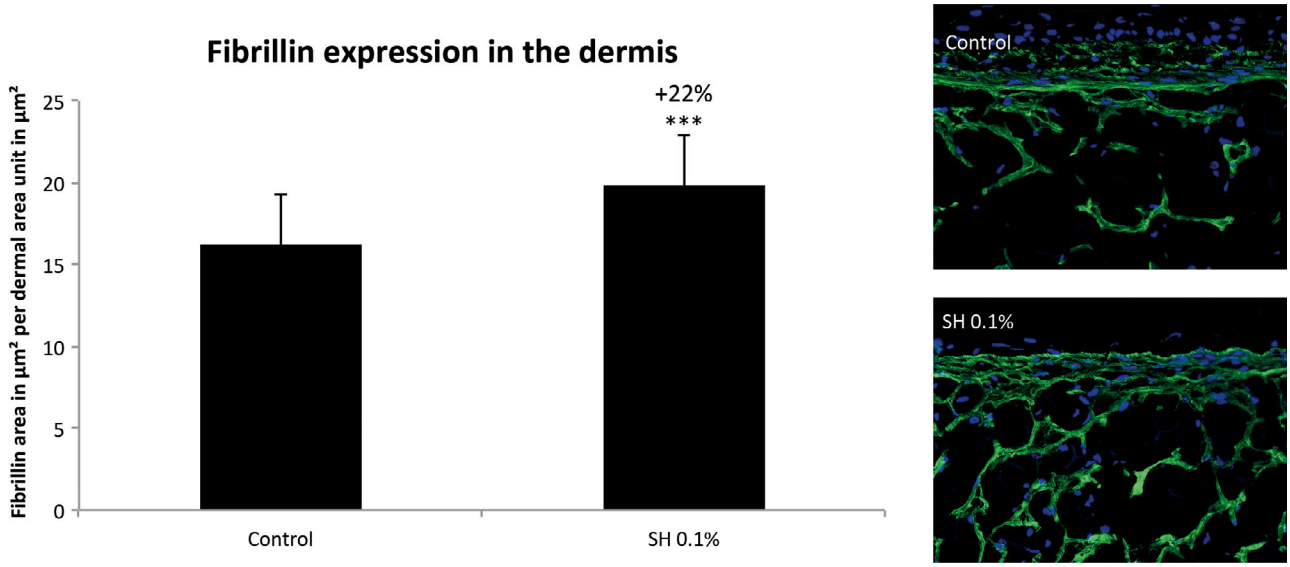


Figure 3 Effect of SH on fibrillin expression on 3D reconstructed full-thickness model. Mean ± SD. ***$P < 0.001$.

A significant improvement of self-esteem has been observed at D56, (+10.5%, $P < 0.001$).

Discussion

Understanding the mechanisms that underlie senescence and skin is of importance not only for cosmetic purposes but also from a biomedical viewpoint. In fact, the susceptibility to specific dermatoses and skin tumours increases with age and has been associated with an increase in the proportion of senescent cells [4]. Therefore, considering the ageing population, there is a fear of a significant increase in cases in the coming years. Furthermore, the abundance of senescent cells also increases in the skin with chronological ageing and photoageing. As shown in normal and BubR1-hypomorphic progeroid mice, preventing the accumulation of p16-expressing cells delays the appearance of a series of traits associated with age [16]. The therapeutic targeting of senescent cells, therefore, seems to be a promising strategy for postponing the onset of these ageing traits. In this context, the search for products in cosmetic and mainly natural products that can reduce senescence induced is being a growing focus in recent years. Many plant extracts have been proposed as having anti-ageing properties. In this study, for the first time, we reported the beneficial effect of an extract from *Sphingomonas hydrophobicum* bacteria acting on senescence, reflecting the potential of environmental bacteria for skin care applications. *Vitreoscilla filiformis* was one of the first bacteria isolated from thermal spa water reported for topical therapy of inflammatory skin disorders [17]. More recently, *Bacillus methylotrophicus* has been proposed as a new type anti-acne preparation to cure or prevent acne [18].

Premature senescence shares some characteristics with replicative or chronologic senescence, such as typical cell morphology, decreased cell proliferation up to irreversible growth arrest or increased SA-β-Galactosidase levels. Based on our results, *Sphingomonas* extract was found to attenuate cellular senescence by suppressing SA-β-galactosidase expression (Fig. 2) and the expression of cell cycle inhibitors (p21 and p16) (Fig. 1A, B). The p21 is a cyclin-dependent kinase (CDK) inhibitor. In consequence of CDK activity suppression, the retinoblastoma protein (pRB) is activated. The p16, another CDK inhibitor, acts through the retinoblastoma (Rb) pathway inhibiting the action of the cyclin-dependent kinases leading to G1 cell cycle arrest [19]. These data might reflect the proliferation-promoting and anti-senescence effects of our *Sphingomonas* extract.

Ageing processes in connective tissues are in large part characterized by extensive disorganization of the extracellular matrix. The physical properties of skin changed dramatically as a function of age, and there are also changes in the amount of macromolecule-associated water in the dermis. As proteoglycans affect the physical properties of tissues and are involved in hydration and resiliency, the age-related changes in skin proteoglycans may contribute to the physical properties of aged skin. Thus, the regulation of proteoglycans and elastic fibre degeneration is essential to prevent the intrinsic skin ageing phenotype. In our study, histological staining indicated increased fibrillin-1 and versican expression after *Sphingomonas* extract treatment (Fig. 4). Versican is a large proteoglycan co-localize with elastic fibres in the dermal compartment [20]. It consists of an amino-terminal globular domain that binds hyaluronan, a central extended region with attached glycosaminoglycans (GAGs) and a carboxy-terminal selectin-like domain (G3) that binds to other matrix components including and fibrillin-1. On one hand, Carino *et al.* [21] compared different age groups ranging from foetal to adult and senescent stages, and noted changes in content and quality of the versican protein including a decrease in versican protein with increasing age. On the other hand, high levels of versican have been shown to protect fibroblasts from apoptosis [22] Based on these data, our results suggest that the increase of versican expression after SH treatment serves to protect fibroblasts from apoptosis, increased proliferation to ensure tissue repair and water uptake.

Besides the *ex vivo* study, we realized an *in vivo* study in order to evaluate the effect of SH extract on skin properties and psychological parameter. Normal skin ageing is characterized by an alteration of the underlying connective tissue with measurable consequences on global skin biophysical properties, and skin relief highly changes. In fact, the cutaneous lines change from a relatively isotropic orientation to a highly anisotropic orientation [23]. This reorganization of the skin relief during the ageing process might be due to a modification of the skin mechanical properties, and particularly to a modification of the dermis mechanical properties. The results of our study indicated that SH extract application during 56 days regained skin flexibility and counteracted the deleterious effect of ageing by significantly increasing isotropy orientation. All the above improve the clinical appearance of patients' skin and conclusively improved their view of their body image and the associated self-esteem, as we noted. Appearance is important in our society and influences the way in which we are perceived by others. The skin is the most visible organ of the body and determines, to a large extent, our appearance. It has been shown that in women, the skin signs of ageing have been perceived as being symptomatic of the loss of femininity, social power and social visibility [24]. As suggested by Baker [7], physical changes between the ages of 50 and 60 years affect one's physical attractiveness and disrupt self-esteem. People with high self-esteem claim to have better relationships and to make better impressions on others.

Conclusion

Research of natural substances that can delay skin ageing has been the object of increasing interest in the last few years [25]. Recently, probiotic bacterial fermentation emerges as one of crucial processing tools in cosmetic technologies in order to enhance absorption into the skin, improve desirable pharmacological activities [26]. In contrast to probiotic extract, little has been done on other microorganisms. To our knowledge, we are the first to identify that *Sphingomonas* extract delays intrinsic skin ageing process by not only attenuate cellular senescence through reduction of the p21 and p16 expression but also increase the expression of fibrillin-1 and versican. Moreover, by acting on biomechanical skin properties, SH extract improved self-esteem. Therefore, *Sphingomonas* extract may have potential therapeutic properties for treating skin ageing, suggesting unique properties of proteobacteria for future investigations on topical applications.

Acknowledgements

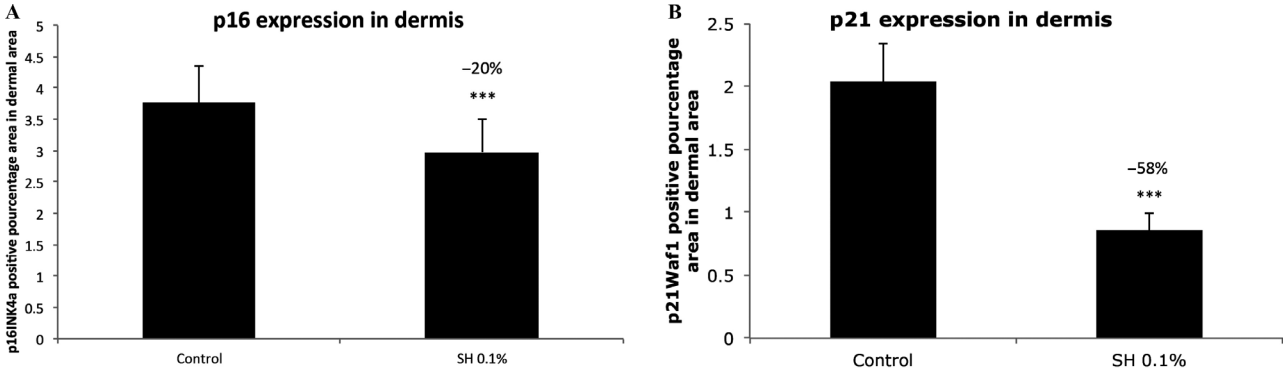
This work was financially supported by GREENTECH SA.

References

1. He, S. and Sharpless, N.E. Senescence in health and disease. *Cell* **169**, 1000–1011 (2017).
2. Lujambio, A. To clear, or not to clear (senescent cells)? That is the question: clearance of senescent cells. *BioEssays* **38**, S56–S64 (2016).
3. Maciel-Barón, L.A., Morales-Rosales, S.L., Aquino-Cruz, A.A., *et al*. Senescence associated secretory phenotype profile from primary lung mice fibroblasts depends on the senescence induction stimuli. *AGE* **38**, 26 (2016).
4. McHugh, D. and Gil, J. Senescence and aging: causes, consequences, and therapeutic avenues. *J. Cell Biol.* **217**, 65–77 (2018).
5. Ritschka, B., Storer, M., Mas, A. *et al*. The senescence-associated secretory phenotype induces cellular plasticity and tissue regeneration. *Genes Dev.* **31**, 172–183 (2017).
6. Velarde, M.C. and Demaria, M. Targeting senescent cells: possible implications for delaying skin aging: a mini-review. *Gerontology* **62**, 513–518 (2016).
7. Baker, L. and Gringart, E. Body image and self-esteem in older adulthood. *Ageing Soc.* **29**, 977–995 (2009).
8. Wang, A.S. and Dreesen, O. Biomarkers of cellular senescence and skin aging. *Front. Genet.* **9**, 977 (2018).
9. Gulati, P., Singh, P., Chatterjee, A.K. and Ghosh, M. Monitoring of biofilm aging in a *Sphingomonas* sp strain from public drinking water sites through changes in capacitance. *Environ. Technol.* **38**, 2344–2351 (2017).
10. Yabuuchi, E., Yano, I., Oyaizu, H., Hashimoto, Y., Ezaki, T. and Yamamoto, H. Proposals of *Sphingomonas paucimobilis* gen. nov. and comb. nov., *Sphingomonas parapaucimobilis* sp. nov., *Sphingomonas yanoikuyae* sp. nov., *Sphingomonas adhaesiva* sp. nov., *Sphingomonas capsulata* comb. nov., and two genospecies of the genus Sphingomonas. *Microbiol. Immunol.* **34**, 99–119 (1990).
11. Takeuchi, M., Kawai, F., Shimada, Y. and Yokota, A. Taxonomic study of polyethylene glycol-utilizing bacteria: emended description of the genus Sphingomonas and new descriptions of *Sphingomonas macrogoltabidus* sp. nov., *Sphingomonas sanguis* sp. nov. and *Sphingomonas terrae* sp. nov. *Syst. Appl. Microbiol.* **16**, 227–238 (1993).
12. Balkwill, D.L., Drake, G.R., Reeves, R.H. *et al*. Taxonomic study of aromatic-degrading bacteria from deep-terrestrial-subsurface sediments and description of *Sphingomonas aromaticivorans* sp. nov., *Sphingomonas subterranea* sp. nov., and *Sphingomonas stygia* sp. nov. *Int. J. Syst. Bacteriol.* **47**, 191–201 (1997).
13. Takeuchi, M., Yoon, J.H., Park, Y.H., Pyun, Y.R., Shin, Y.K. and Lee, J.S. *Sphingomonas aquatilis* sp. nov., *Sphingomonas koreensis* sp. nov., and *Sphingomonas taejonensis* sp. nov., yellow-pigmented bacteria isolated from natural mineral water. *Int. J. Syst. Evol. Microbiol.* **51**, 1491–1498 (2001).
14. Alessandri, G., Vecchione, M., Eisenberg, N. and Łaguna, M. On the factor structure of the Rosenberg (1965) general self-esteem scale. *Psychol. Assess.* **27**, 621–635 (2015).
15. Twenge, J.M. and Campbell, W.K. Self-esteem and socioeconomic status: a meta-analytic review. *Personal. Soc. Psychol. Rev.* **6**, 59–71 (2002).
16. Baker, D.J., Childs, B.G., Durik, M. *et al*. Naturally occurring p16Ink4a-positive cells shorten healthy lifespan. *Nature* **530**, 184–189 (2016).
17. Volz, T., Skabytska, Y., Guenova, E. *et al*. Nonpathogenic bacteria alleviating atopic dermatitis inflammation induce IL-10-producing dendritic cells and regulatory Tr1 cells. *J. Invest. Dermatol.* **134**, 96–104 (2014).
18. Choi, U.-H., Nam, E.S., Ahmad, R.I. and Park, Y.-H. In vitro evaluation of bioactive potential of Bacillus methylotrophicus YML008 against Propionibacterium acnes. *Pak. J. Pharm. Sci.* **29**, 1081–1085 (2016).
19. Dolan, D.W.P., Zupanic, A., Nelson, G. *et al*. Integrated stochastic model of DNA damage repair by non-homologous end joining and p53/p21- mediated early senescence signalling. *PLoS Comput. Biol.* **11**, e1004246 (2015).
20. Bernstein, E.F., Fisher, L.W., Li, K., LeBaron, R.G., Tan, E.M. and Uitto, J. Differential expression of the versican and decorin genes in photoaged and sun-protected skin. Comparison by immunohistochemical and northern analyses. *Lab Investig. J. Tech. Meth. Pathol.* **72**, 662–669 (1995).
21. Carrino, D.A., Sorrell, J.M. and Caplan, A.I. Age-related changes in the proteoglycans of human skin. *Arch. Biochem. Biophys.* **373**, 91–101 (2000).
22. Sheng, W., Wang, G., Wang, Y. *et al*. The roles of versican V1 and V2 isoforms in cell proliferation and apoptosis. *Mol. Biol. Cell* **16**, 1330–1340 (2005).
23. Pailler-Mattei, C., Debret, R., Vargiolu, R., Sommer, P. and Zahouani, H. In vivo skin biophysical behaviour and surface topography as a function of ageing. *J. Mech. Behav. Biomed. Mater.* **28**, 474–483 (2013).
24. Honigman, R. and Castle, D.J. Aging and cosmetic enhancement. *Clin. Interv. Aging* **1**, 115–119 (2006).
25. Ortiz-Espín, A., Morel, E., Juarranz, Á. *et al*. An extract from the plant *Deschampsia antarctica* protects fibroblasts from senescence induced by hydrogen peroxide. *Oxid. Med. Cell Longev.* **2017**, 1–16 (2017).
26. Shin, D., Lee, Y., Huang, Y.-H. *et al*. Probiotic fermentation augments the skin antiphotoaging properties of *Agastache rugosa* through up-regulating antioxidant components in UV-B-irradiated HaCaT keratinocytes. *BMC Complement Altern. Med.* **18**, 196 (2018).

Graphical Abstract

The contents of this page will be used as part of the graphical abstract of html only. It will not be published as part of main.



Using a physiological approach, we investigated whether bacteria extract can have a beneficial effect of skin senescence markers. Our results show that Sphingomonas extract may have potential therapeutic properties for treating skin ageing, suggesting unique properties of proteobacteria for future investigations on topical applications.